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Wiersma, P.; Tinbergen, J.M.

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NO NOCTURNAL ENERGETIC SAVINGS IN RESPONSE TO HARD WORK IN FREE-LIVING GREAT TITS

by

POPKO WIERSMA and JOOST M. TINBERGEN

(*Zoological Laboratory, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands. E-mail: p.wiersma@biol.rug.nl*)

ABSTRACT

We measured energy expenditure in free-living great tits (*Parus major*) during the active (day) and the inactive period (night) with the aim of determining whether great tits compensate for energy costs made during periods of high activity in periods of low activity. If such compensation occurs, inferences from measurements of energy expenditure over a 24 h period, with regard to the energy costs of the different behavioural elements, may obscure relations between parental effort and energy expenditure. Also, energy budgets, based on estimates of time budgets combined with a fixed cost for each behavioural category observed, may be unreliable if animals are able to compensate for energy costs made during periods of high activity in periods of low activity.

Laboratory studies have revealed a reduction in resting metabolic rate (RMR) when birds are forced to work harder during the day, but it has not yet been investigated whether such nocturnal savings are also made under natural conditions.

We manipulated brood size in a free-living population of great tits to create a difference in the demands of the nest, measured effort (feeding visits) and daily energy expenditure (DEE). In order to test whether compensation occurred we measured both DEE over 24 h, and resting metabolic rates (RMR) of female great tits at night. DEE and feeding rate differed between the experimental groups, being higher in females rearing enlarged broods, but we did not find evidence of nocturnal saving.

KEY WORDS: reproductive effort, energy savings, brood size manipulation, resting metabolic rate, daily energy expenditure

INTRODUCTION

It has recently been discovered that birds, when pressed to work for their food, reduced night-time energy expenditure. Evidence of such energy compensation comes from laboratory studies on zebra finches (*Taeniopygia guttata*) and common starlings (*Sturnus vulgaris*), by DEERENBERG *et al.* (1998) and BAUTISTA *et al.* (1998), respectively. They made birds work for food, during a period of *ca* 4 weeks, and showed that nocturnal mass-specific metabolic rate was reduced when activity was experimentally increased. These findings may have repercussions for life history studies.

Life history theory hinges crucially on the premise that organisms make costs in order to reproduce. The price of reproduction is paid for through a declining future reproductive output. The theory considers parental effort, the work parents do for their current offspring, as the reason for this declining future reproductive output. However, effort has many dimensions, since it represents anything the parents do for their current offspring. Empiricists have, for a long time, investigated factors limiting reproductive output. As a starting point, parental effort was substituted by feeding rate but more recently by parental energy expenditure. Stimulated by the work of HAILS & BRYANT (1979) and DRENT & DAAN (1980) the research concentrated on the question whether and how energy expenditure limits reproductive output.

Empirical studies have concentrated on the effect of brood size manipulation on energy expenditure, with the expectation that energy expenditure would increase with brood size. However, correlations between energy expenditure and brood size are sometimes much less pronounced than expected, or even absent (TINBERGEN & VERHULST, 2000; VERHULST & TINBERGEN, 1997; WRIGHT *et al.*, 1998). WILLIAMS (1987), HAILS & BRYANT (1979) and MORENO *et al.* (1995) could only show a correlation between feeding rate and DEE in one of the sexes. In his overview, BRYANT (1988) shows that five out of nine studies cannot detect a positive relationship between energy expenditure and nest visit rate. Also MORENO (1989) could not detect any effect on energy expenditure of brood size manipulation in both parents in northern wheatears (*Oenanthe oenanthe*). TINBERGEN & VERHULST (2000) questioned the role of energy expenditure on the cost of reproduction. They could not show a difference in female DEE between the control and enlarged broods, whereas birds with enlarged broods did show a lower probability of starting a second clutch. Therefore, they concluded that DEE *per se* is probably not causally involved in the cost of reproduction.

We argue that empirical studies of the effect of brood size on energy expenditure (discussed above) may fail to find a relationship due to night-time compensation of increased work rate during the active phase. Hence, estimates of parental work rate based on energy expenditure measurements over a 24 h period, as taken by doubly-labelled water, may give misleading results. Moreover, as mentioned by DEERENBERG *et al.* (1998), translation of time budgets to energy budgets will be inadequate if night-time compensation occurs.

Published studies of night-time compensation were carried out in the laboratory, which may not mimic adequately the natural conditions with respect to the possibilities the birds have to adjust their energy budgets. Therefore, we decided to study the occurrence of night-time reduction of

energy expenditure in response to increased daytime work in the field. We chose a well-known system; great tits caring for offspring. Work rate of the parents, as measured by provisioning rate and DEE, can, in the study population chosen, be altered by brood size manipulation (SANZ & TINBERGEN, 1999). We measured night-time energy expenditure using oxygen consumption, and from the same individuals we estimated the 24 h energy turnover using doubly-labelled water.

METHODS

The experiment was carried out in 1997 and 1998 in the Lauwersmeer, located in the north of the Netherlands. Four woods were used, consisting of plots of varying sizes containing either coniferous or deciduous trees. Coniferous plots were usually bordered by deciduous trees and shrubs. In total 200 nestboxes were present. The boxes were fixed to the trees at a height of *ca* 2 m.

Nestboxes were checked regularly during the breeding season to assess clutch size, first day of incubation, number of hatchlings and fledglings, and date of hatching and fledging. During the nestling period, when the chicks were around 10 days old, attempts were made to catch both parents using a spring trap placed inside the nestbox. All nestlings and newly caught adults were ringed and morphometric measurements were taken. For tarsus length we measured the length of the tibiotarsus with callipers (resolution 0.1 mm) and the length of the third primary was measured using a stopped ruler (1 mm) (SVENSSON, 1992). Body mass was measured with a spring balance (0.1 g).

We manipulated the brood size by adding or removing three chicks when they were 2 days old. Triplets of matching nests were selected on the basis of hatching date (maximum difference 1 day), clutch size (maximum difference two eggs) and location. Thirteen nest pairs were in the same wood, and four pairs were in separate, but nearby, woods. Chicks were moved between two of the nests, leaving one nest serving as a control. For the metabolic measurements presented in this paper only the nests with reduced and enlarged broods were used. In total, we took measurements of 17 pairs of nests. Figures concerning timing of the breeding season and numbers of eggs and nestlings are given in table 2.

The resting metabolic rate was measured when the nestlings were on average 12 days old. DEE of female parents, and feeding rates of both parents, were measured, in 1997 only, immediately prior to, or after the RMR measurement.

At night, between 22:00 and 00:00, two birds were caught in their nestboxes and weighed. They were held in a respirometer until sunrise,

i.e., 04:00-04:45, and then returned to their nestboxes. In 1997, the oxygen consumption rate was first measured at ambient temperature (average 14.9°C, range 10.1-20.3) and after *ca* 2 h the temperature was increased to, on average, 23.4°C (range 19.6-27.1). In 1998, only a measurement at a high temperature was taken (average 27.8°C, range 23.5-30.4). The high temperatures varied due to differences in ambient temperature and battery capacity. The respirometer boxes were darkened with black plastic sheets. Although it was difficult to ascertain whether the birds were stressed during the measurements, we have some indications that they were not. A few times the birds were seen sleeping in the boxes when these were uncovered again after measurement. At other times, when this was not observed, we cannot be sure if they had been sleeping or not, because the disturbance of uncovering the box might have alarmed and woken them. Strong activity can be seen from erratic changes to the oxygen consumption readings. There were always long periods of steady values in the measurements, indicating that the bird inside the box was not moving much.

When the bird could be caught quickly, body temperature was measured with a microthermistor connected to a datalogger (Squirrel 1259, Grant Instruments, Cambridge), inserted *ca* 1.5 cm in the cloaca (accuracy 0.2°C). When the time it took to insert the thermistor and take a reading was too long (more than 10 s) to yield a reliable measurement, the data were omitted from analyses.

In 1997 only, daily energy expenditure of female parents was measured using doubly-labelled water (D_2O^{18}, further referred to as DLW). The females were caught in the nestbox during the night following the day that the chicks were 11 days old (range 10-13). The birds were injected intraperitoneally with 0.10 or 0.12 ml DLW and an initial blood sample was taken from a brachial vein 1 h later. Twenty-four h later we tried to recapture the bird to take the final blood sample. A detailed description of the DLW technique used can be found in TINBERGEN & DIETZ (1994). Because frequently we did not succeed in recapturing the birds 24 h later (see results), some birds were caught again with a trap early the next morning. In these cases, the time interval over which energy expenditure was measured was longer and to ensure acceptable accuracy in the analyses we changed from injecting with 0.10 to 0.12 ml of DLW. The measurements over longer time intervals were corrected by linear interpolation to a 24 h period. We corrected for the proportion of inactive time in the measurement period, assuming the birds were inactive from 20:40-05:00 h (own obs., and see TINBERGEN & DIETZ, 1994), and assuming that the metabolic rate in the active period was 1.96 times that in the inactive period (TINBERGEN & DIETZ, 1994). DLW analyses were

carried out at the Centre for Isotope Physics (University of Groningen, The Netherlands). For the calculations we assumed that the body water content was 66% (MERTENS, 1987) and that the RQ was 0.75.

In the beginning of the 1997 season, the oxygen measurement was performed immediately after the final DLW-blood sample was taken. However, due to the disturbance, frequently the birds were not present in the nestbox that night, and a final blood sample could not be taken, or only after trying to trap the bird the following morning. We then chose to do the oxygen measurement immediately after the DLW injection and prior to taking the initial blood sample. This ensured that we had the oxygen measurement at least.

Feeding activity was observed directly with video cameras. In the morning following the DLW injection the nestbox visits were recorded for 3 h. The cameras were positioned *ca* 10 m from the nestbox, preferably in or behind a bush and covered as well as possible with leaves and grass. The recordings were analysed to determine the feeding rate of both male and the female.

Nocturnal metabolism measurements were made with a two-channel, car-battery powered, transportable respirometer. The portable oxygen analyser (Servomex model 570A, Crowborough, UK) had an accuracy of $\pm 0.1\%$ (manufacturer's specification). The measurement accuracy is, in fact, higher because the difference between the measured channels and a reference channel (outside air) was used instead of an absolute value. The zero value of the oxygen analyser was calibrated with nitrogen gas ($<0.001\%$ O_2) before, and, in 1997 only, after each measurement. Outside air (assumed 20.95% O_2) was used to calibrate the so-called span of the meter. In 1997 we used a floating ball flow meter (Sho-Rate model 1355, Brooks Instrument, Veenendaal, The Netherlands) with an accuracy of 0.2 l/h (manufacturer's specification). These meter readings were adjusted to standard temperature and pressure. In 1998, mass flow controllers were used (model 5860S, Brooks Instrument) with an accuracy of 0.12 l/h (manufacturer's specification). Air flow rates through the respirometer chambers (volume 1.7 l) were, on average, 19 l/h in 1997 and, owing to deploying mass flow meters, 12 l/h in 1998. Because at night the birds were probably mainly assimilating fat, an RQ of 0.75 and an energy equivalent of the O_2 consumption rate of 19.83 kJ/l (SCHMIDT-NIELSEN, 1997) were assumed. As RMR value we took the lowest point from a stable period of measurements.

Ambient temperature and the temperature inside the respirometer chamber (T_{box}) were recorded continuously with thermistors (accuracy 0.2°C, manufacturer's specification). O_2-, flow- (in 1998 only) and temperature values were recorded every minute with a digital data logger (Squirrel

1259). The temperature inside the respirometer boxes could be increased by putting power on a high capacity resistor fixed to the lid on the inside of the box.

Unfortunately, skipping the calibration at the end of the respirometer measurements in 1998 resulted in incomparable data sets. This was caused by a non-linear drift of the meter output, probably due to temperature changes inside the oxygen analyser. In fact, the oxygen consumption measured in 1998 would be on average 11% lower when applying a correction factor calculated from the 1997 calibration data. The variation in this factor is, however, too large to make accurate corrections. The average correction factor corresponds well with the actual difference between years (9%, table 1). Consequently, comparison of data from the two years is not possible. Since two females of an experimental triad were measured simultaneously, time related measurement biases were avoided.

Variation in nocturnal energy expenditure was analysed using residuals of RMR, further called RMR_{resid}, as measured at the high temperatures. These were calculated using backward stepwise regression analysis, entering variables for which we did not control fully in the experimental set-up, *i.e.*, tarsus length, third primary length, respirometer box temperature, body mass and year. This resulted in a model that included box temperature and year as best predicting variables, with a r^2 of 0.35 (table 1). The fact that temperature was still affecting the metabolic rate means that the birds were not within the thermoneutral zone at all (high) temperatures. Neither the squared value of T_{box} (to check for a curvilinear relationship), nor the interaction between year and T_{box} added significantly to the model.

TABLE 1

Results of regression analysis with resting metabolic rate (W), or mass specific resting metabolic rate (W/g), as measured at the high temperatures, as dependent variable. T_{box} is the temperature inside the respirometer chamber (°C) and year has a value of 0 (1997) or 1 (1998).

	$b \pm SEM$	t_{41}	P
Dependent variable: RMR			
Intercept	0.522 ± 0.074	7.02	<0.0001
T_{box}	-0.00836 ± 0.00315	−2.65	<0.05
year	0.0877 ± 0.0191	−4.61	<0.0001
dependent variable: SRMR			
Intercept	0.0273 ± 0.0043	6.25	<0.0005
T_{box}	-0.000369 ± 0.004365	−2.00	0.052
year	0.00393 ± 0.00019	−3.51	<0.005

Mass specific RMR (W/g), further called SRMR, as measured at high temperatures, was also related to respirometer box temperature and varied between years ($r^2 = 0.24$, table 1).

Values following the means and parameter estimates are the standard errors.

RESULTS

Effects on broods

There were no differences in reproductive parameters between the manipulated pairs before the manipulation was performed (table 2). After the manipulation, when the nestlings were 7 days old, the brood size was 6.2 in the reduced broods and 11.8 in the enlarged broods. The mass difference between enlarged and reduced broods was 79.5 g (74% of the reduced brood mass). Also in each separate year the brood masses differed (t-tests, 97: $t_{19} = -9.13$, $P < 0.0005$; 98: $t_{14} = -9.98$, $P < 0.0005$). More birds fledged from the enlarged broods, although mortality was higher in the latter (paired t-test: $t_{16} = -8.0$, $P < 0.0005$). On average 1.7 nestlings died between 0-14 days old (day of hatching = 0) in an enlarged brood, and 0.2 in the reduced.

The body mass of the individual nestlings, at 14 days old, was higher in the reduced broods than in the enlarged broods (table 3). Wing and tarsus length was not significantly different. When comparing the data of the 2 years, no difference in the effect of the manipulation on body mass can be shown (ANOVA on mass differences and with year as factor: $F_{1,33} = 0.04$, $P = 0.8$).

Because the combined DLW and O_2 measurements meant elaborate handling of the birds we could imagine that their behaviour would be

TABLE 2

Means and standard errors of various characters of the experimental broods in 1997 and 1998. Reduction and enlargement of broods was performed at nestling age of 1 day, and consisted of a transfer of three individuals. Test statistics resulted from paired t-tests. Only those broods are included of which also respirometry measurements were collected.

	Reduced	Enlarged	t_{16}	P
Hatching date (day in May)	17.9 ± 2.4	18.0 ± 2.4	−1.46	0.16
Clutch size	9.9 ± 0.4	9.8 ± 0.4	0.32	0.75
Hatchling number	9.3 ± 0.3	9.0 ± 0.4	0.86	0.40
Nestling number (day 7)	6.2 ± 0.3	11.8 ± 0.4	−14.8	<0.0005
Brood mass (day 14; g)	106.5 ± 5.3	186.0 ± 7.4	−14.5	<0.0005
Number fledged	6.1 ± 0.3	10.3 ± 0.6	−7.98	<0.0005

TABLE 3

Mean body measurements of nestlings, averaged per brood, at nestling age of 14 days, from enlarged and reduced broods. Experimental nests of which no energetic measurements were collected are included. Standard error of means between brackets. Statistics result from paired sample t-tests.

	Reduced	Enlarged	t	P
1997 ($n = 20$ pairs)				
body mass	17.25 ± 0.33	16.29 ± 0.36	2.19	0.042
wing length	33.84 ± 0.45	33.80 ± 0.62	0.54	0.96
tarsus length	19.69 ± 0.10	19.63 ± 0.11	0.45	0.66
1998 ($n = 15$ pairs)				
body mass	17.49 ± 0.19	16.41 ± 0.40	2.68	0.018
wing length	32.63 ± 0.63	32.21 ± 0.75	0.49	0.63
tarsus length	19.76 ± 0.08	19.60 ± 0.13	1.09	0.30

changed as a consequence, resulting in lower feeding rates and lower growth rates of their young. Therefore, the mass of these nestlings was compared with those of unmeasured parents. For young of unmeasured parents, a selection of the data with the same range of hatching dates and nestling numbers as the nestlings with measured parents was used. This selection yielded 74 broods, while 25 broods with a measured parent were available. The body masses of nestlings 14 days old were not significantly affected by the energy measurements of a parent (general linear model with mean nestling mass in brood as dependent factor and controlling for number of nestlings and date of hatch: $F_{1,95} = 1.79$, $P = 0.18$).

Feeding rates

Feeding rates were measured in 1997. In order to avoid effects of age differences, a selection of pairs was made in which both broods of a pair were of exactly the same age before testing the difference (14 out of 17). The rates were found to be higher in enlarged broods both in females and in males (table 4). The difference between the sexes in their reaction to the manipulation was tested by comparing the differences between reduced and enlarged broods in feeding rates between males and females (paired t-test: $t_8 = 1.19$, $P = 0.27$), and by testing the relative differences in feeding rates between reduced and enlarged broods (paired t-test: $t_8 = 1.08$, $P = 0.3$). Thus, no difference between the sexes was apparent, in accordance with previous studies.

TABLE 4

Average feeding rates with standard errors of means of female, male and both parents, with experimentally reduced or enlarged brood sizes. The rates were measured as the number of nest visits per hour and originate from single 3 h observations. The age of the nestlings at the measurement was on average 11.6 ± 0.12 days. The test statistics were based on paired sample *t*-tests.

Category	Reduced	Enlarged	t_{13}	P
Female	10.1 ± 2.88	22.8 ± 4.20	−6.72	<0.001
Male	12.4 ± 2.04	20.4 ± 2.82	−2.12	0.054
Both parents	22.4 ± 2.76	43.2 ± 4.92	−4.54	<0.01

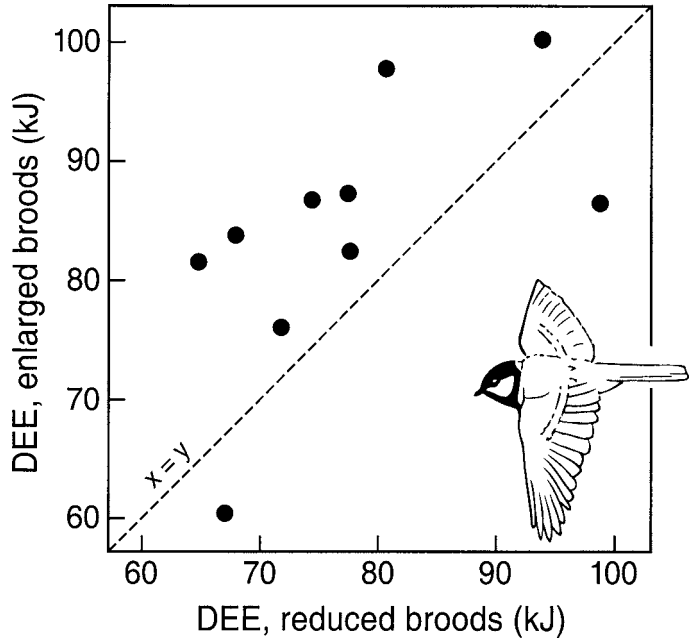

Fig. 1. Daily energy expenditure of females tending reduced or enlarged broods with nestling of 11 days old. Each dot represents data of two females of a manipulation pair. The dotted line represents the line of equality.

DEE

Average daily energy expenditures over 24 h as measured with DLW of females with reduced or enlarged broods were 77.5 ± 3.6 kJ/d and 84.2 ± 3.5 kJ/d, respectively. A paired *t*-test showed an almost significant effect of the brood size manipulation ($t_9 = -2.12$, $P = 0.063$, fig. 1,

TABLE 5

Results of energy measurements on adult female great tits tending a reduced or enlarged brood. RMR_{pred} stands for RMR adjusted to the year 1997 and adjusted for respirometer box temperature. All other abbreviations are explained in the text. All test statistics were based on paired sample *t*-tests.

Category	Reduced	Enlarged	*t* (df)	*P*
DEE (kJ/d)	77.5 ± 3.6	84.2 ± 3.5	-2.12 (9)	0.063
RMR_{pred} (W)	0.347 ± 0.0079	0.353 ± 0.0088	-1.17 (16)	0.3
RMR_{resid} (W)	0.00231 ± 0.0107	0.00193 ± 0.00888	0.04 (16)	1.0
$SRMR_{resid}$ (W/g)	$3.07 \times 10^{-4} \pm 6.20 \times 10^{-4}$	$-9.20 \times 10^{-5} \pm 5.81 \times 10^{-4}$	0.57 (16)	0.6
			0.57 (16)	0.6
EE_{ρ} (kJ)	11.7 ± 0.79	12.2 ± 0.79	-0.53 (6)	0.6
EE_{α} (kJ)	64.1 ± 4.0	72.0 ± 5.5	-2.52 (6)	0.045
body mass (g)	17.5 ± 0.20	18.0 ± 0.22	-1.47 (16)	0.16

table 5). Body masses of the birds did not differ between the two categories (table 5).

RMR and mass specific RMR

Because at a certain point we changed the order of DLW and oxygen consumption measurements (see methods section), we first tested whether this had affected the outcome of RMR. This does not seem to be the case (General Linear Model, controlling for T_{box}: $F_{1,12} = 0.45$, $P = 0.51$).

RMR_{resid} (see table 1) were used for the pairwise comparison of the two manipulation categories (fig. 2, table 5). The paired RMR_{resid} did not differ significantly (mean reduced $= 0.00231 \pm 0.0107$ W, enlarged $= 0.00193 \pm 0.00888$ W, $t_{16} = 0.04$, $P = 0.97$). The average of both residuals does not equal zero, because they were calculated using a more extensive data set. The average RMR which the regression model (table 1) predicts, adjusted to the average values of 1997, are 0.347 ± 0.0078 W and 0.353 ± 0.0087 W, respectively (table 5). Also in each separate year there was no difference (97: $t_7 = -0.50$, $P = 0.6$, 98: $t_8 = 0.20$, $P = 0.8$). A power analysis shows that the difference between the two groups could maximally be 0.034 W ($\alpha = 0.05$, power $= 0.80$, SE of difference $= 1.113$, $n = 17$) (BUCHNER *et al.*, 1997). This is about 11% of BMR.

The paired residuals of $SRMR_{resid}$ (see table 1) did not differ either. The mean value for the reduced broods was $3.07 \times 10^{-4} \pm 6.20 \times 10^{-4}$ W/g, and for the enlarged $-9.20 \times 10^{-5} \pm 5.81 \times 10^{-4}$ W/g ($t_{16} = 0.57$, $P = 0.6$; table 5). Also in each separate year there was no difference (97: $t_7 = -0.17$, $P = 0.9$, 98: $t_8 = 0.79$, $P = 0.5$).

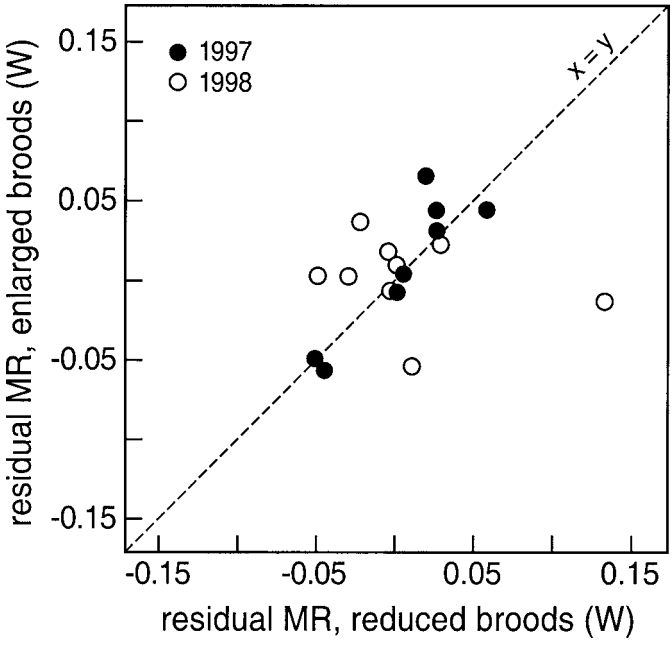

Fig. 2. Residual metabolic rates of adult female great tits during the nestling period. Residuals result from a linear regression with minimum metabolic rate as dependent variable and the temperature of the respirometer box and year as predicting variables. Each dot represents a paired sample of a bird attending a reduced or an enlarged brood.

Day and night time metabolism

Even if RMR is unchanged, the energy expenditure over longer periods may differ, *e.g.*, due to an earlier decline, or delayed rise of metabolic rate. Therefore, we also analysed variation in the amount of energy expended during the entire resting phase. We did so by using the metabolism measurements at the low temperatures (in 1997 only), which are very close to ambient temperature; T_{box} was on average 1.3 ± 0.52°C higher than the actual ambient temperature. A correction was made to predict RMR at the ambient temperature, according to the next equation:

$$EE_{\rho} = RMR * (T_{body} - T_{a\rho})/(T_{body} - T_{box}) * 3.6 * 8.34$$

This equation assumes a constant heat conductance value and body temperature (Scholander curve). Body temperature (T_{body}) was set at 39.42°C (average of 18 cloacal measurements), $T_{a\rho}$ is the ambient temperature during the dark period of the DLW measurement, and T_{box} is the temperature

in the respirometer box when RMR was measured. The factor 3.6 is applied to convert Watts to kJ/h, and this was multiplied by 8.34, the average length of the inactive period in hours (TINBERGEN & DIETZ, 1994). This yields a value for the total amount of kJ spent during the night. Subtracting this from the DLW measurement of DEE gave an estimate of the amount of energy spent during the active period (EE_α). EE_ρ is a very conservative estimate because the birds will for part of the night have a metabolic rate above the resting value, *e.g.*, due to heat increment of feeding.

The total energy expenditure during the night was not correlated with the energy expenditure during the day ($r = -0.35$, $n = 16$, $P = 0.18$; Fig. 3). In a paired test the day- and night-time energy expenditure of females rearing reduced or enlarged broods were compared. After selection of the paired samples (*i.e.*, with both DLW and O_2 measurements performed without problems) seven pairs remained. EE_ρ did not differ between the manipulation categories ($t_6 = -0.53$, $P = 0.6$), while EE_α did ($t_6 = -2.52$, $P = 0.045$; table 5). EE_α of females with reduced broods was 64.1 ± 4.0 kJ, and with enlarged broods 72.0 ± 5.5 kJ. EE_ρ of

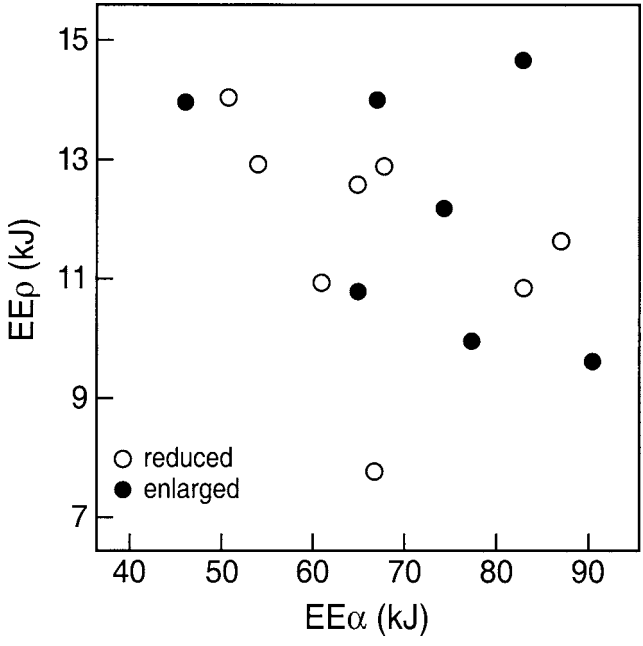

Fig. 3. Relation between energy expenditure during the active phase (EE_α) and the resting phase (EE_ρ). Active phase expenditure is calculated as the DLW data minus an estimate of nocturnal energy expenditure based on the oxygen measurements and the air temperature.

females with reduced broods was 11.7 ± 0.8 kJ and with enlarged broods 12.2 ± 0.79 kJ.

DISCUSSION

The average DEE values measured here are 12% lower than those measured in great tits in the same area in 1995 by SANZ & TINBERGEN (1999). They found that female great tits with nestlings of 12 days old, rearing reduced broods, expended on average 86.3 ± 4.95 kJ/d ($n = 5$) and when rearing enlarged broods they expended 98.4 ± 6.36 kJ/d ($n = 4$). In our study the expenditure of females with reduced and enlarged broods was, respectively, 77.5 kJ and 84.2 kJ. We tested whether the difference between the two studies was significant, using a general linear model, with DEE of the reduced and enlarged broods treated as repeated measures, and with the two studies entered as factor. It showed that the results from the two studies do not differ significantly ($F_{1,12} = 3.26$, $P = 0.096$), and that the overall effect of manipulation is significant ($F_{1,12} = 8.97$, $P = 0.011$). The average DEE of both studies combined are 79.54 ± 3.07 for the reduced, and 88.26 ± 3.47 for the enlarged broods ($n = 14$).

The average feeding rates of females reported by SANZ & TINBERGEN (1999) from the same area in 1995 were higher, *i.e.*, 19.7 ± 3.0 ($n = 10$) and 34.7 ± 2.7 ($n = 9$) visits per brood per hour for reduced and enlarged broods, respectively. In this study it was 10.1 ± 2.9 ($n = 13$) and 22.8 ± 4.2 ($n = 13$) visits per brood per hour (t-test, reduced: $t_{21} = 2.27$, $P = 0.03$, enlarged: $t_{20} = 2.15$, $P = 0.04$). Therefore, the tendency to lower DEE values found in this study may be the result of lower feeding rates. The most obvious causes for the lower feeding rates are food-related factors and temperature differences.

The average RMR we measured at high temperatures in 1997 was 0.325 ± 0.011 W ($n = 24$). Because, at least, some values were measured below the thermoneutral zone, BMR will be lower. We could only find one study (HISSA & PALOKANGAS, 1970) giving RMR values of great tits. They showed that the thermoneutral zone starts above 28°C, and that the metabolic rate, in summer, at temperatures between 29 and 33°C, is 0.37 W. This value is considerably higher than ours, more so considering the higher temperatures at which they measured. The difference may be related to the higher latitude of their study site (60°31′ *vs* 53°06′). BMR according to allometric predictions of KENDEIGH *et al.* (1977) is 0.307 ± 0.003 W ($n = 41$), for a 17.3 g passerine bird in the resting phase in summer. The mean of our measured value of RMR is not different from the predicted value (t-test, $t_{63} = 1.95$, $P = 0.056$).

As a result of the brood size manipulation, the parent birds had to feed more nestlings in the enlarged broods (table 2). Although the growth of the nestlings was negatively affected by the brood size change (table 3), the rate at which the nestlings were fed by the female parent was 2.3 times higher in the enlarged broods (table 4). As was the purpose of our experiment, parents with enlarged broods put more effort, measured as visits per hour, in feeding their young.

In accordance with the increased provisioning rate, DEE was 1.086 times (*i.e.*, 6.7 kJ/d) higher in female parents with enlarged broods (fig. 1). No effect on metabolic rates, either RMR (fig. 2) or mass specific RMR, and on night-time energy expenditure (fig. 3) could be shown. Evidence for compensation for elevated energy output during the working period is therefore lacking.

Correlative analyses do not give any indication of reduced RMR in response to higher feeding efforts either. There was no correlation between the feeding rates of the females and their resting metabolic rate ($r = 0.42$, $n = 20$, $P = 0.07$). Although this value is close to significance, omission of a single point, with a very high feeding rate, weakens the correlation considerably. Temperature corrected SRMR is positively correlated with the feeding rate of the females ($r = 0.45$, $n = 20$, $P = 0.045$). But when a single outlier is omitted from the analysis it is not significant anymore ($r = 0.22$, $n = 19$, $P = 0.4$).

In spite of the fact that the feeding rates of the females are positively related to DEE (regression coefficient $= 30.0 \pm 11.3$, $t_{19} = 2.64$, $P = 0.016$, where feeding rate is expressed in number per minute and DEE in kJ), the relationship between EE_ρ and feeding rate is not significant ($t_{14} = 0.88$, $P = 0.4$), while it is almost significant for EE_α (regression coefficient $= 28.5 \pm 14.2$, $t_{14} = 2.01$, $P = 0.064$).

The lack of evidence for nocturnal energy savings in the great tit could be related to the small scope there may be for any substantial savings on a daily basis because nights lasted only 8.5 h. Zebra finches reduced RMR by 18% when faced with a high workload and, all else being equal, and assuming BMR is reduced during the resting phase only, this would yield a saving of 2.8-4.2% on a daily basis (depending on the estimate of DEE used; DEERENBERG *et al.* (1998)). Starlings faced with a high workload reduced their BMR by 35.4%, which would yield a reduction of 9.5% on a daily basis (BAUTISTA *et al.*, 1998). The working period was 14 h in the zebra finches and only 8 h in the starlings, which explains part of the outcomes in total savings in the two studies. If the BMR reduction were applicable during the whole day, DEE would be, respectively, 8.4-12.7% and 16.2% lower.

When applying these figures to great tits, reducing RMR during the resting phase, would result in a 2.3% (zebra finch) to 4.6% (starling)

reduction of DEE. Assuming that the BMR reduction is applicable during the whole day, these values are 6.5 and 12.8%, respectively. These reductions are in all cases below those estimated in zebra finches and starlings, but overall are fairly similar. Hence, the lack of scope for energy savings does not seem to be the explanatory factor. The most influential factor in reducing DEE in the zebra finches was apparently the decrease in activity during the day, when not foraging. Perhaps this is not a feasible option for the wild great tits tending a brood. The starling data show that substantial savings can be made through a reduction of RMR. However, these birds had also reduced their body mass considerably, namely by 18%. The decline in body mass in the zebra finches was only 3%. The great tits did not show a difference in body mass between the manipulation categories (17.5 ± 0.20 *vs* 18.0 ± 0.22 g for reduced and enlarged broods, respectively; table 5).

Another cause for a lack of compensation in great tits could be that their intensity of energy output, *i.e.*, scaled to their species specific BMR, was lower than the values in the other two studies. BMR of zebra finches is 0.21 W (own measurements), of great tits 0.31 W, or somewhat lower (this study), and of starlings 0.76 W (starlings in 'easy' condition in BAUTISTA *et al.*, 1998). The DEE values of these species in the three studies were *ca* 2.8, 2.9 and $2.5 \times$BMR, respectively. These values are very close and, therefore, it cannot be the case that differences in DEE, corrected for BMR, give rise to the difference in results.

It is not immediately clear what causes the difference in results between this study and the studies of BAUTISTA *et al.* (1998) and DEERENBERG *et al.* (1998). It is possible that the lab studies suffer from unnatural feeding conditions. The set-up of the systems was such that the birds did not experience any random variation in feeding success. This may result in cognitive constraints ('demotivation'), which would reduce foraging activity (FOTHERINGHAM, 1998). If this is actually the case then those birds were starved, and starvation may lead to mass reduction of metabolic active tissue, and subsequently to a decline in BMR (DAAN *et al.*, 1989; PIERSMA *et al.*, 1996).

Another possible cause which may explain the lack of any effect on BMR of the brood size manipulation could be the effect of the annual phase the birds were in. During the reproductive phase, other decisions, including physiological ones, may be taken, because fitness costs of lowering RMR may vary.

There is also the possibility that the time allowed for accommodation to the hard-working situation was too short in the great tit experiment. The manipulation was performed when the nestlings were 2 days old and the measurements were done 10 days later. In the experiments of

DEERENBERG *et al.* (1998) and BAUTISTA *et al.* (1998) the birds had more time to accustom themselves to the work levels to which they were submitted, *i.e.*, at least 3 weeks.

We conclude that there are no indications that free-living great tits save energy by reducing their nocturnal energy expenditure in response to an increased energy expenditure after brood enlargement. The use of DEE as an estimate of parental work load in field studies remains valid.

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